

CERTIFICATION OF MAILING

CERTIFICATION UNDER 37 CFR 1.10

Express Mail Number: EL 897 621 483 015 Date of Deposit: October 24, 2001

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


Mila T. Kasan

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Lee et al.

Attorney Docket No. 4478D2

Serial No: not yet assigned

Art Unit:

Filed: concurrently herewith

Examiner:

Title: WATER-SOLUBLE RHODAMINE DYE CONJUGATES

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Washington, D. C. 20231

Sir:

Applicants submit the Preliminary Amendment concurrently with this application.

I. AMENDMENTS

Prior to examination on the merits, please enter the following amendments to the above-identified patent application. No new matter has been added to the specification.

This is a preliminary amendment under 37 C.F.R. 1.53(b) for a division of Application No. 09/661,206, filed on Sept. 14, 2000, now pending, which is a division of Application No. 09/433,093, filed on November 3, 1999, now US Patent No. 6,191,278, issued Feb. 20, 2001, all of which are incorporated herein by reference. This priority statement has been included on page 1 of the specification filed herein.

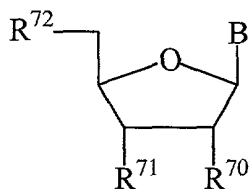
In the claims:

Please cancel claims 1-69 without prejudice.

Please add new claims 70-105 as follows:

-- 70. A labeled nucleoside/tide or nucleoside/tide analog comprising a rhodamine dye conjugated by a linker to a nucleoside/tide or nucleoside/tide analog, wherein:

the rhodamine is a rhodamine-type parent xanthene having attached to the xanthene C9 carbon a phenyl group that is further substituted with an ortho carboxy or ortho sulfonate group or a salt thereof, one to three substituted or unsubstituted aminopyridinium groups and a substituted or unsubstituted alkylthio, or arylthio group; and the nucleoside/tide or nucleoside/tide analog comprises the structure:

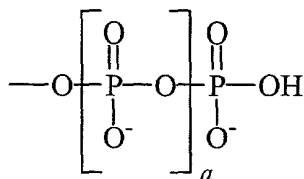


wherein:

B is a nucleobase selected from a purine, a 7-deazapurine, an 8-aza,7-deazapurine, a pyrimidine, a normal nucleobase and a common analog of a normal nucleobase;

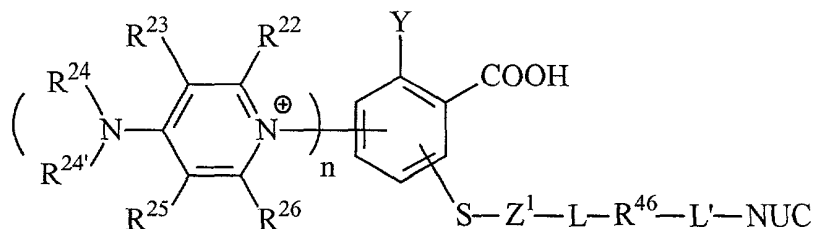
R^{70} and R^{71} , when taken alone, are each independently selected from hydrogen, hydroxyl and a moiety which blocks polymerase-mediated template-directed polymerization, or when taken together form a bond such that the illustrated sugar is 2',3'-didehydroribose; and

R^{72} is selected from hydroxyl, a phosphate ester having the formula:



where a is an integer from 0 to 2, and a phosphate ester analog, or a salt thereof.

71. The labeled nucleoside/tide or nucleoside/tide analog of claim 70 comprising the formula:



wherein:

Y is a rhodamine-type parent xanthene ring attached to the illustrated phenyl group at the xanthene C9 carbon;

R^{22} , R^{23} , R^{25} , and R^{26} are independently selected from hydrogen and (C₁-C₆) alkyl;

R^{24} , when taken alone, is (C₁-C₆) alkyl, or when taken together with $R^{24'}$ is (C₄-C₁₀) alkylidyl, (C₄-C₆) alkyleno, (C₄-C₆) heteroalkylidyl and (C₄-C₆) heteroalkyleno;

$R^{24'}$, when taken alone, is (C₁-C₆) alkyl, or when taken together with R^{24} is (C₄-C₁₀) alkylidyl, (C₄-C₆) alkyleno, (C₄-C₆) heteroalkylidyl and (C₄-C₆) heteroalkyleno;

n is 1, 2, or 3;

S is sulfur;

Z¹ is selected from (C₁-C₁₂) alkylidyl, (C₁-C₁₂) alkylidyl independently substituted with one or more of the same or different W¹ groups, (C₅-C₁₄) arylidyl, and (C₅-C₁₄) arylidyl independently substituted with one or more of the same or different W² groups;

W¹ is selected from -X, -R, =O, -OR, -SR, =S, -NRR, =NR, -CX₃, -CN, -OCN, -SCN, -NCO, -NCS, -NO, -NO₂, =N₂, -N₃, -S(O)₂O⁻, -S(O)₂OH, -S(O)₂R, -C(O)R, -C(O)X, -C(S)R, -C(S)X, -C(O)OR, -C(O)O⁻, -C(S)OR, -C(O)SR, -C(S)SR, -C(O)NRR, -C(S)NRR and -C(NR)NRR;

W² is selected from -R, -OR, -SR, -NRR, -S(O)₂O⁻, -S(O)₂OH, -S(O)₂R, -C(O)R, -C(O)X, -C(S)R, -C(S)X, -C(O)OR, -C(O)O⁻, -C(S)OR, -C(O)SR, -C(S)SR, -C(O)NRR, -C(S)NRR and -C(NR)NRR;

L is selected from a bond, (C₁–C₁₂) alkyldiyl, (C₁–C₁₂) substituted alkyldiyl, (C₆–C₂₆) arylalkyldiyl, –O–, –S–, –NR–, –C(O)O–, –C(O)NR–, –NRS(O)₂–, –NR–NR–, –NRC(O)O–, and –NRC(O)NR–;

R⁴⁶ is selected from –C(O)NR–, –C(O)O–, and –C(O)S–,

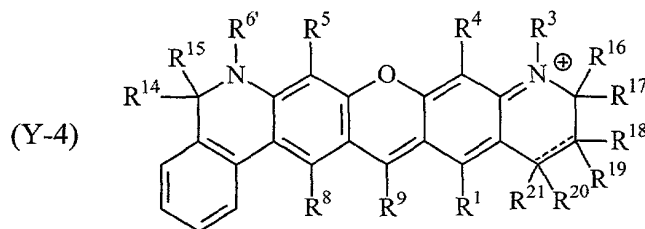
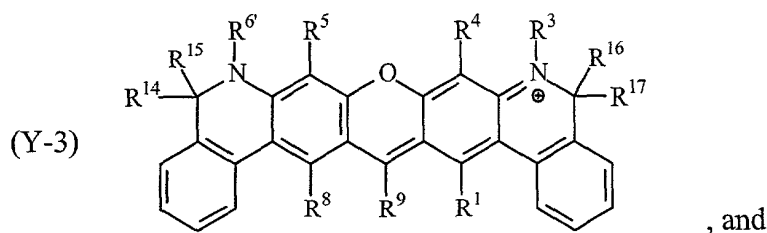
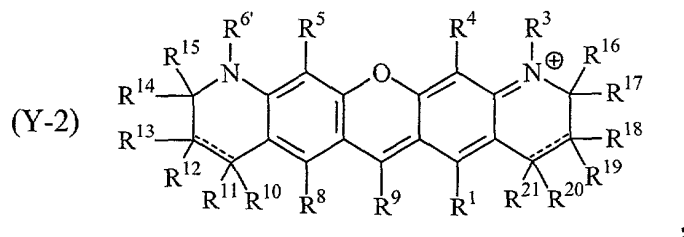
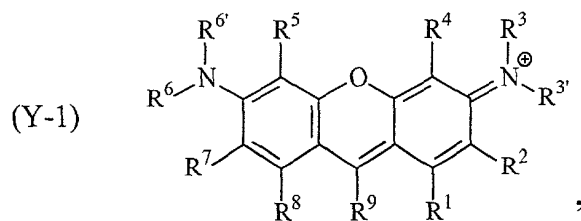
L' is selected from (C₁–C₂₀) alkyldiyl, (C₁–C₂₀) heteroalkyldiyl, (C₁–C₂₀) alkyleno, (C₁–C₂₀) heteroalkyleno, (C₆–C₂₆) arylalkyldiyl, (C₅–C₂₀) heteroarylalkyldiyl, and substituted forms thereof; and

NUC is a nucleoside/tide or nucleoside/tide analog;

each R is independently selected from hydrogen, (C₁–C₆) alkyl, (C₅–C₂₀) aryl, (C₆–C₂₆) arylalkyl, and (C₅–C₂₀) arylaryl; or when two R groups on the same nitrogen atom are taken together, those two R groups are (C₄–C₁₀) alkyldiyl or (C₄–C₁₀) alkyleno; and

each X is independently a halogen.

72. The labeled nucleoside/tide or nucleoside/tide analog of claim 71 wherein Y comprises the rhodamine-type parent xanthene ring structures:



and a salt thereof, wherein:

R^1 and R^2 when taken alone, are independently hydrogen or (C_1-C_6) alkyl;

R^3 and $R^{3'}$ when taken alone, are independently selected from hydrogen, (C_1-C_6) alkyl, (C_5-C_{14}) aryl and (C_5-C_{14}) arylaryl, or when taken together is (C_4-C_6) alkyldiyl or (C_4-C_6) alkyleno, or when individually taken together with R^2 or R^4 is (C_2-C_6) alkyldiyl or (C_2-C_6) alkyleno;

R^4 , when taken alone, is selected from hydrogen and (C_1-C_6) alkyl, or when taken together with R^3 or $R^{3'}$ is (C_2-C_6) alkyldiyl or (C_2-C_6) alkyleno;

R^5 , when taken alone, is selected from hydrogen and (C_1-C_6) alkyl, or when taken together with R^6 or $R^{6'}$ is (C_2-C_6) alkyldiyl or (C_2-C_6) alkyleno;

R^6 and $R^{6'}$ when taken alone, are selected from hydrogen, (C_1-C_6) alkyl, (C_5-C_{14}) aryl and arylaryl, or when taken together are (C_4-C_6) alkylidyl or alkyleno, or when individually taken together with R^5 or R^7 is (C_2-C_6) alkylidyl or alkyleno;

R^7 , when taken alone, is selected from hydrogen and (C_1-C_6) alkyl, or when taken together with R^6 or $R^{6'}$ is (C_2-C_6) alkylidyl or alkyleno;

R^8 , when taken alone, is selected from hydrogen and (C_1-C_6) alkyl;

R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , R^{18} , R^{19} , R^{20} and R^{21} are each independently selected from hydrogen and (C_1-C_6) alkyl, or

when R^{10} , R^{11} , R^{12} and R^{13} taken together are (C_5-C_{14}) aryleno or (C_5-C_{14}) aryleno substituted with one or more of the same or different (C_1-C_6) alkyl, or

when R^{18} , R^{19} , R^{20} and R^{21} taken together are (C_5-C_{14}) aryleno or aryleno substituted with one or more of the same or different (C_1-C_6) alkyl; and

R^9 is the point of attachment to the xanthene C9 carbon.

73. The labeled nucleoside/tide or nucleoside/tide analog of claim 72 wherein R^2 when taken together with R^3 or $R^{3'}$ is (C_2-C_6) alkylidyl or (C_2-C_6) alkyleno.

74. The labeled nucleoside/tide or nucleoside/tide analog of claim 72 wherein:
an alkylidyl or alkyleno bridge formed by taking R^2 together with R^3 or $R^{3'}$, R^7 together with R^6 or $R^{6'}$, or R^4 together with and R^3 or $R^{3'}$, is ethano, propano, 1,1-dimethylethano, 1,1-dimethylpropano or 1,1,3-trimethylpropano;

an aryleno bridge formed by taking R^1 together with R^2 is benzo or naphtho;

an alkylidyl or alkyleno bridge formed by taking R^3 together with $R^{3'}$, or R^6 together with $R^{6'}$, is butano;

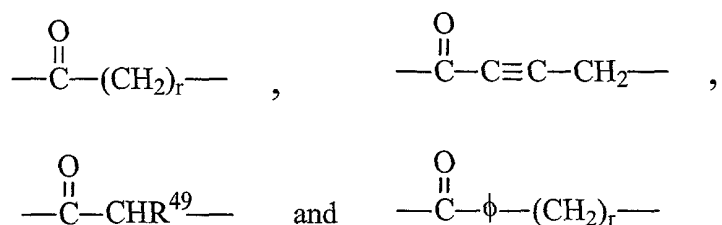
an alkylidyl or alkyleno bridge formed by taking R^5 together with R^6 or $R^{6'}$ is ethano, propano, 1,1-dimethylethano, 1,1-dimethylpropano and 1,1,3-trimethylpropano; and

an aryleno bridge formed by taking R^{10} , R^{11} , R^{12} and R^{13} together, or R^{18} , R^{19} , R^{20} and R^{21} together, is benzo.

75. The labeled nucleoside/tide or nucleoside/tide analog of claim 71 in which Z¹ is phenyldiyl.

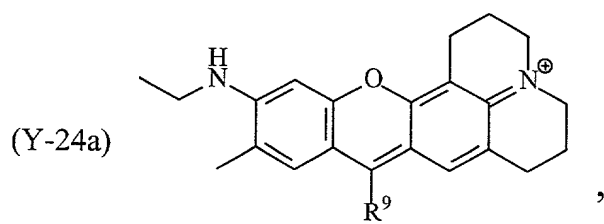
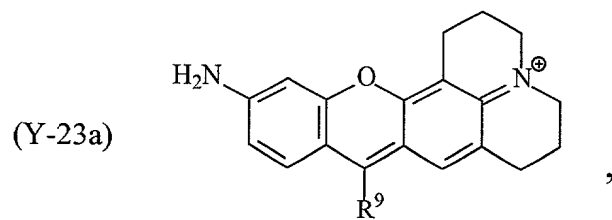
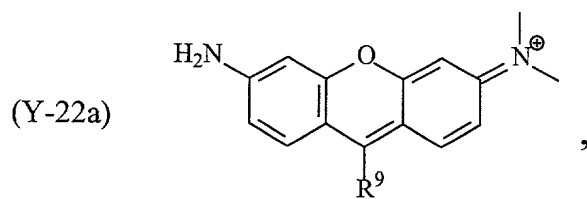
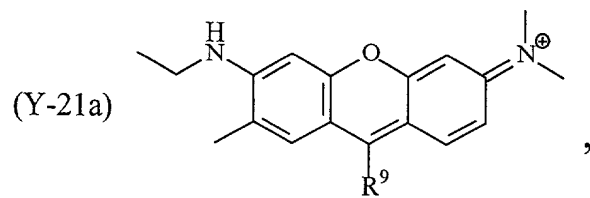
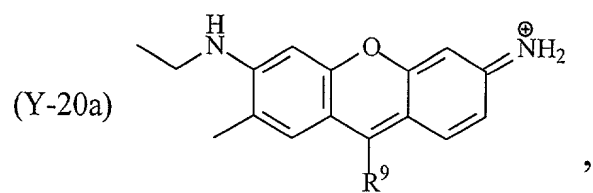
76. The labeled nucleoside/tide or nucleoside/tide analog of Claim 71 in which L' is selected from: $\text{—C}\equiv\text{C—CH}_2\text{—}$ and $\text{—C}\equiv\text{C—CH}_2\text{—O—CH}_2\text{CH}_2\text{—}$.

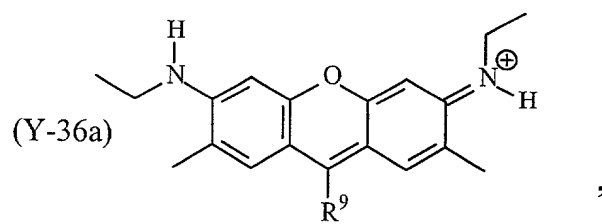
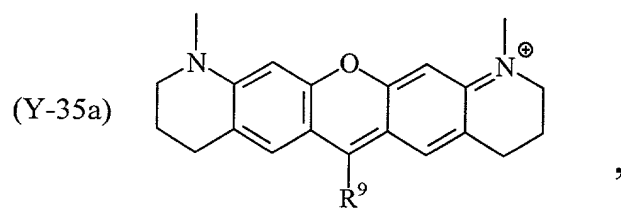
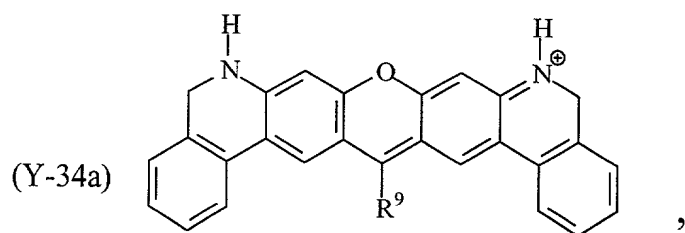
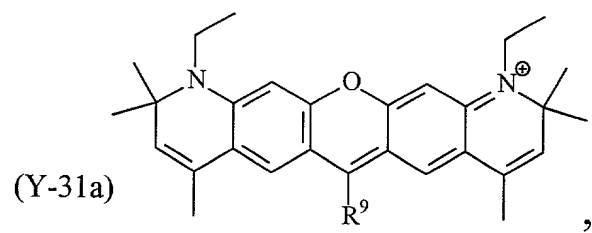
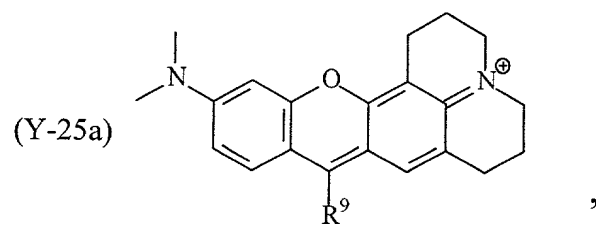
77. The labeled nucleoside/tide or nucleoside/tide analog of Claim 71 in which L' is: $\text{—C}\equiv\text{C—CH}_2\text{—O—CH}_2\text{CH}_2\text{—N}^{\text{R}^{47}}\text{—R}^{48}\text{—}$ wherein R⁴⁷ is hydrogen or (C₁–C₆) alkyl, and R⁴⁸ is selected from:

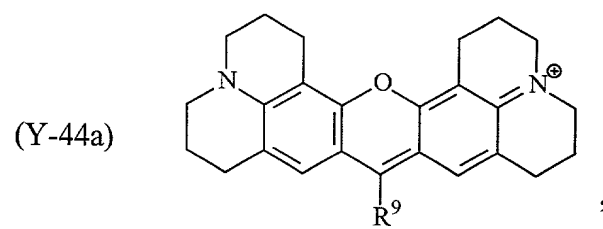
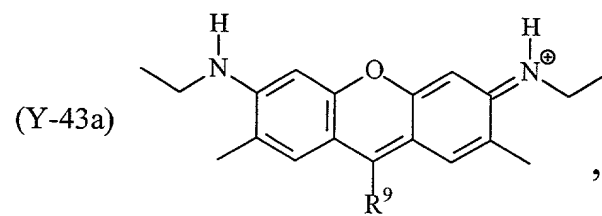
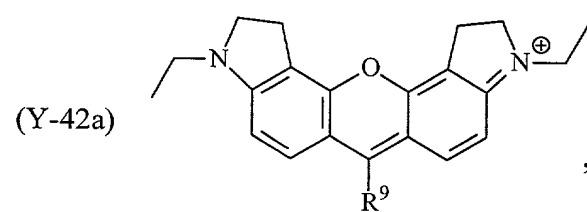
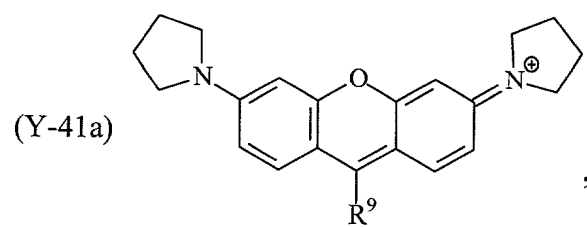
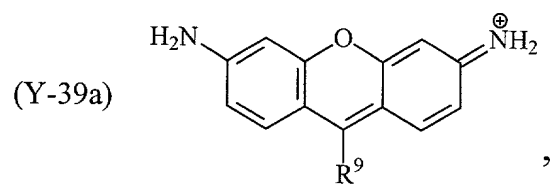


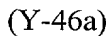
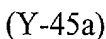
wherein each r is independently an integer from 1 to 6; R⁴⁹ is hydrogen, (C₁–C₆) alkyl, or an amino acid side chain; and ϕ is phenyldiyl or substituted phenyldiyl.

78. The labeled nucleoside/tide or nucleoside/tide analog of claim 71 in which Y is selected from the structures:

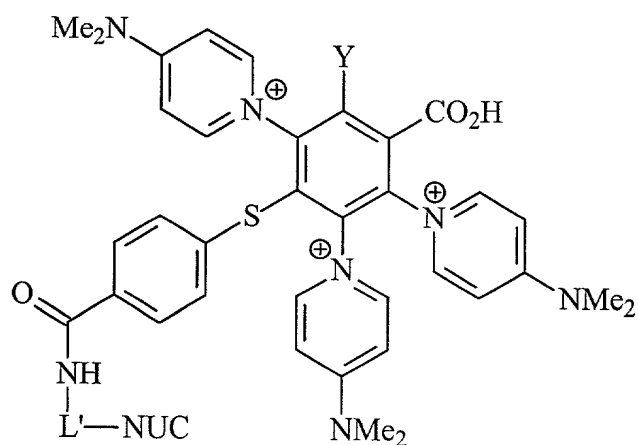






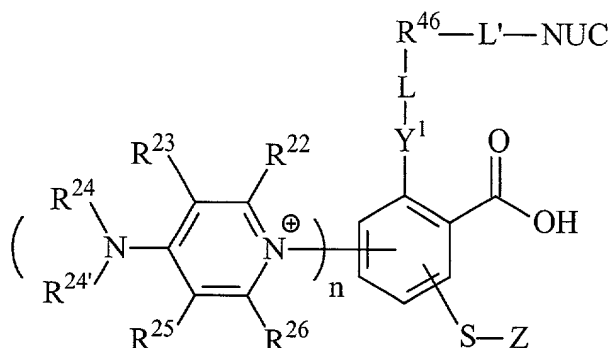


80. The labeled nucleoside/tide or nucleoside/tide analog of claim 71 which comprises the structure:



81. The labeled nucleoside/tide or nucleoside/tide analog of Claim 80 in which L' is selected from: $\text{—C}\equiv\text{C—CH}_2\text{—}$ and $\text{—C}\equiv\text{C—CH}_2\text{—O—CH}_2\text{CH}_2\text{—}$

28644_1



wherein:

Y^1 is a rhodamine-type parent xanthene ring attached to the illustrated phenyl group at the xanthene C9 carbon;

R^{22} , R^{23} , R^{25} , and R^{26} are independently selected from hydrogen and (C_1-C_6) alkyl;

R^{24} , when taken alone, is (C_1-C_6) alkyl, or when taken together with $R^{24'}$ is (C_4-C_{10}) alkyldiyl, (C_4-C_6) alkyleno, (C_4-C_6) heteroalkyldiyl or (C_4-C_6) heteroalkyleno;

$R^{24'}$, when taken alone, is (C_1-C_6) alkyl, or when taken together with R^{24} is (C_4-C_{10}) alkyldiyl, (C_4-C_6) alkyleno, (C_4-C_6) heteroalkyldiyl or (C_4-C_6) heteroalkyleno;

n is 1, 2, or 3;

S is sulfur;

Z is (C_1-C_{12}) alkyl, (C_1-C_{12}) alkyl substituted with one or more of the same or different W^1 groups, (C_5-C_{20}) aryl, and (C_5-C_{20}) aryl substituted with one or more of the same or different W^2 groups;

W^1 is selected from $-X$, $-R$, $=O$, $-OR$, $-SR$, $=S$, $-NRR$, $=NR$, $-CX_3$, $-CN$, $-OCN$, $-SCN$, $-NCO$, $-NCS$, $-NO$, $-NO_2$, $=N_2$, $-N_3$, $-S(O)_2O^-$, $-S(O)_2OH$, $-S(O)_2R$, $-C(O)R$, $-C(O)X$, $-C(S)R$, $-C(S)X$, $-C(O)OR$, $-C(O)O^-$, $-C(S)OR$, $-C(O)SR$, $-C(S)SR$, $-C(O)NRR$, $-C(S)NRR$ and $-C(NR)NRR$;

W^2 is selected from $-R$, $-OR$, $-SR$, $-NRR$, $-S(O)_2O^-$, $-S(O)_2OH$, $-S(O)_2R$, $-C(O)R$, $-C(O)X$, $-C(S)R$, $-C(S)X$, $-C(O)OR$, $-C(O)O^-$, $-C(S)OR$, $-C(O)SR$, $-C(S)SR$, $-C(O)NRR$, $-C(S)NRR$ and $-C(NR)NRR$;

L is a selected from a bond, (C_1-C_{12}) alkyldiyl, (C_1-C_{12}) substituted alkyldiyl, (C_6-C_{26}) arylalkyldiyl, $-O-$, $-S-$, $-NR-$, $-C(O)O-$, $-C(O)NR-$, $-NRS(O)_2-$, $-NR-NR-$, $-NRC(O)O-$, and $-NRC(O)NR-$;

R^{46} is selected from $-C(O)NR-$, $-C(O)O-$, and $-C(O)S-$,

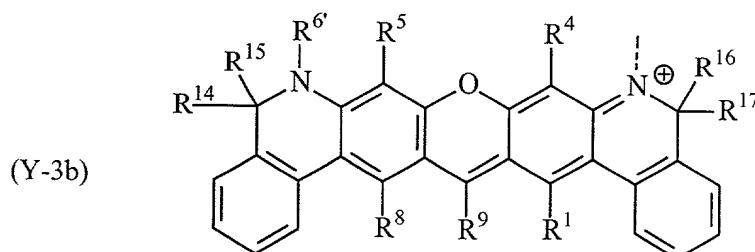
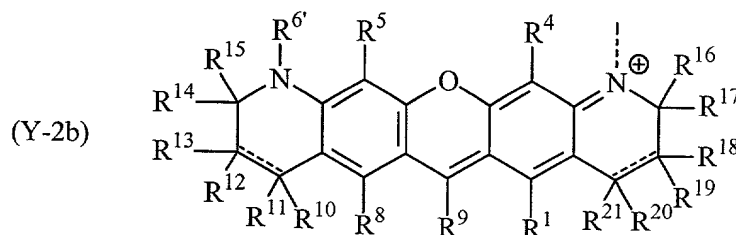
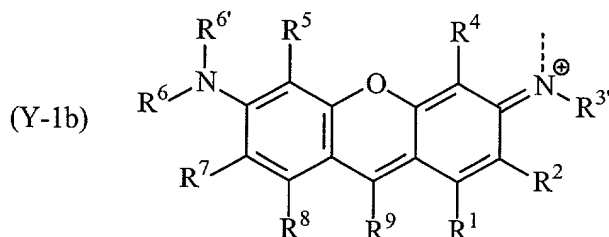
L' is selected from (C_1-C_{20}) alkylidyl, (C_1-C_{20}) heteroalkylidyl, (C_1-C_{20}) alkylene, (C_1-C_{20}) heteroalkylene, (C_6-C_{26}) arylalkylidyl, (C_5-C_{20}) heteroarylalkylidyl, and substituted forms thereof; and

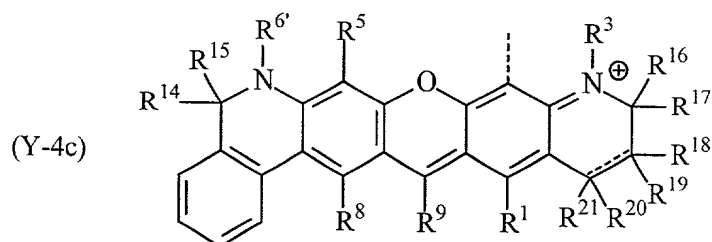
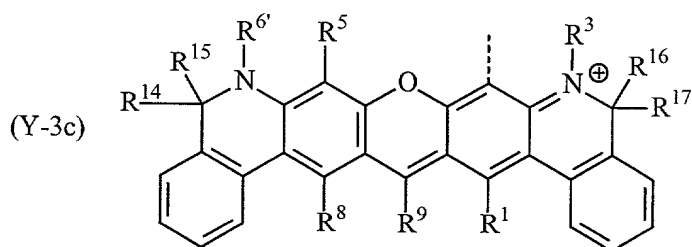
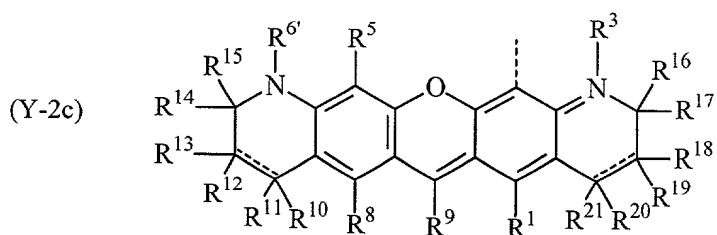
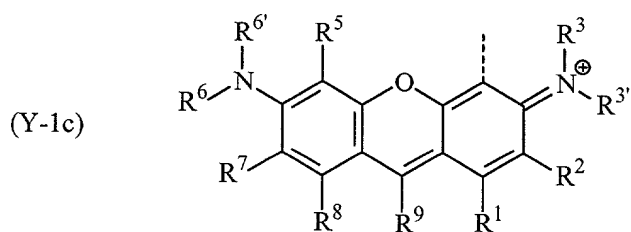
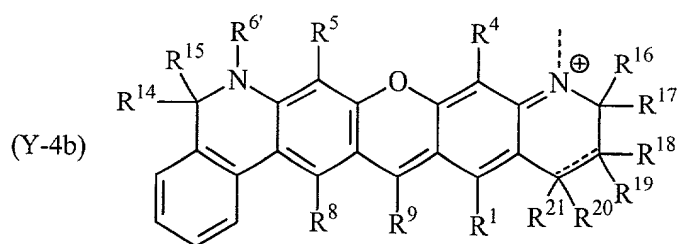
NUC is a nucleoside/tide or nucleoside/tide analog;

each R is independently selected hydrogen, (C_1-C_6) alkyl, (C_5-C_{20}) aryl, (C_6-C_{20}) arylalkyl, and (C_6-C_{20}) arylaryl; or when two R groups on the same nitrogen atom are taken together, those two R groups are (C_4-C_{10}) alkylidyl or (C_4-C_{10}) alkylene; and

each X is independently a halogen.

83. The labeled nucleoside/tide or nucleoside/tide analog of Claim 82 in which Y^1 is selected from:





wherein the dashed line at the nitrogen or C4 atom indicates the point of attachment of L.

84. The labeled nucleoside/tide or nucleoside/tide analog of claim 82 wherein:

an alkylidiyl or alkylene bridge formed by taking R^2 together with R^3 , R^4 together with R^3 , R^5 together with R^6 , or R^7 together with R^6 , is ethano, propano,

1,1-dimethylethano, 1,1-dimethylpropano or 1,1,3-trimethylpropano; and

an aryleno bridge formed by taking R^{10} , R^{11} , R^{12} and R^{13} together or R^{18} , R^{19} , R^{20} and R^{21} together is benzo.

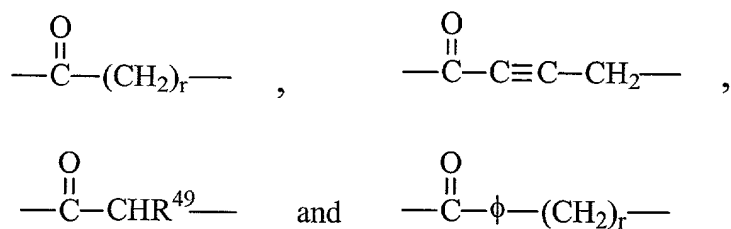
85. The labeled nucleoside/tide or nucleoside/tide analog of claim 82 in which L is selected from phenylidiyl and naphthylidiyl.

86. The labeled nucleoside/tide or nucleoside/tide analog of claim 82 in which L is $-(CH_2)_i-\phi-$ where i is an integer from 1 to 6 and ϕ is phenylidiyl or naphthylidiyl.

87. The labeled nucleoside/tide or nucleoside/tide analog of claim 82 in which Z is selected from phenyl, benzyl, naphthyl, pyridyl and purinyl.

88. The labeled nucleoside/tide or nucleoside/tide analog of Claim 82 in which L' is selected from: $-\text{C}\equiv\text{C}-\text{CH}_2-$ and $-\text{C}\equiv\text{C}-\text{CH}_2-\text{O}-\text{CH}_2\text{CH}_2-$.

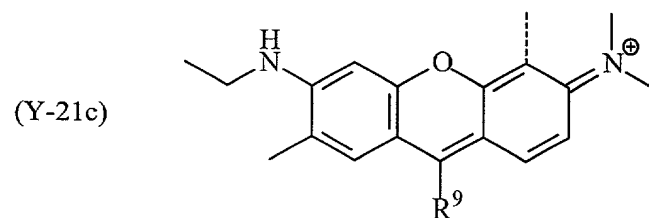
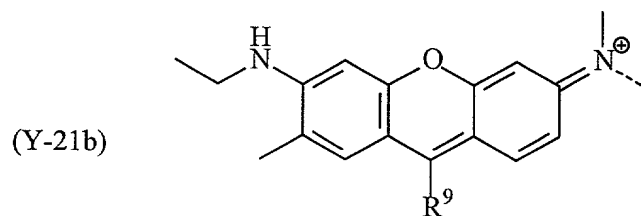
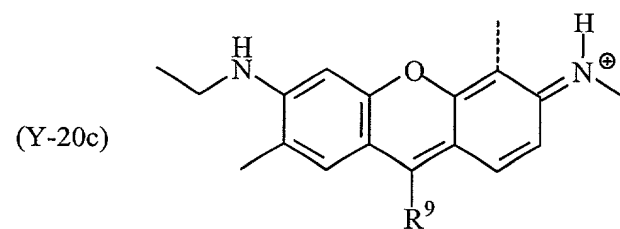
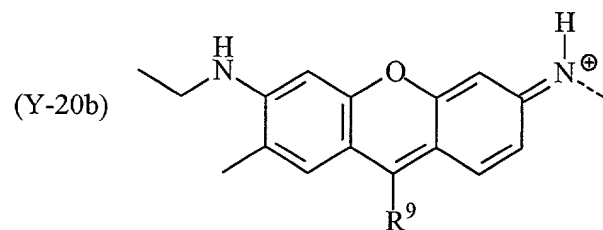
89. The labeled nucleoside/tide or nucleoside/tide analog of Claim 82 in which L' is: $-\text{C}\equiv\text{C}-\text{CH}_2-\text{O}-\text{CH}_2\text{CH}_2-\overset{\text{R}^{47}}{\underset{|}{\text{N}}}-\text{R}^{48}-$ wherein R^{47} is hydrogen or (C_1-C_6) alkyl, and R^{48} is selected from:

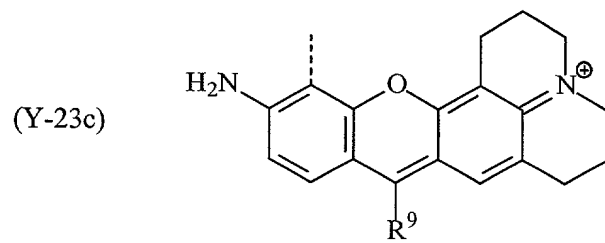
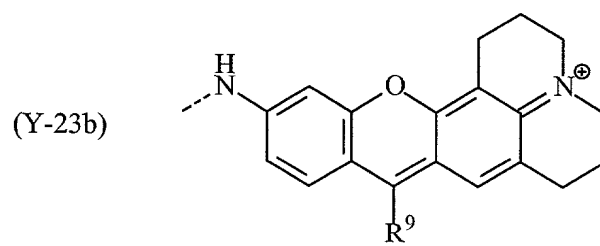
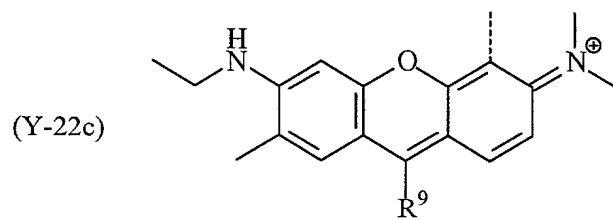
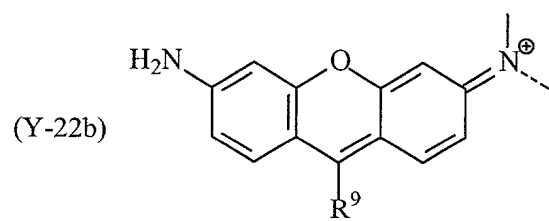


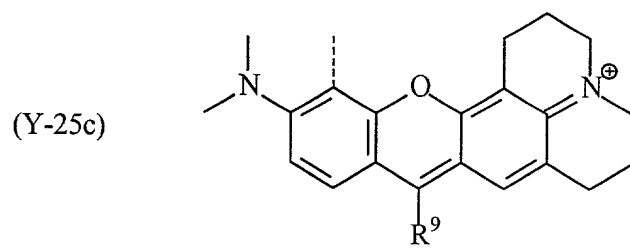
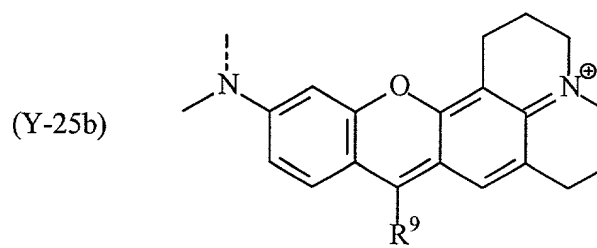
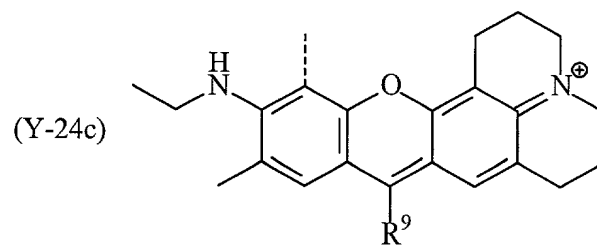
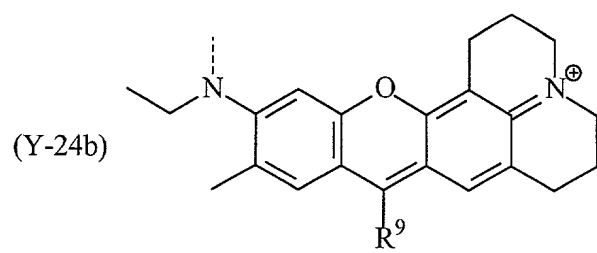
wherein each r is independently an integer from 1 to 6; R^{49} is hydrogen, (C_1-C_6) alkyl, or an amino acid side chain; and ϕ is phenylidiyl or substituted phenylidiyl.

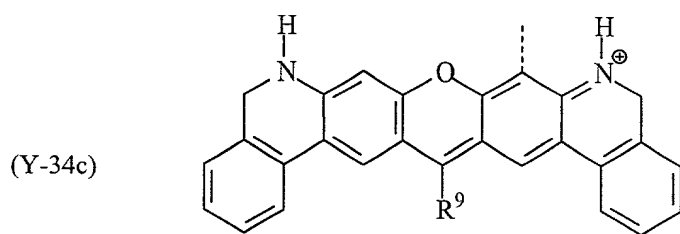
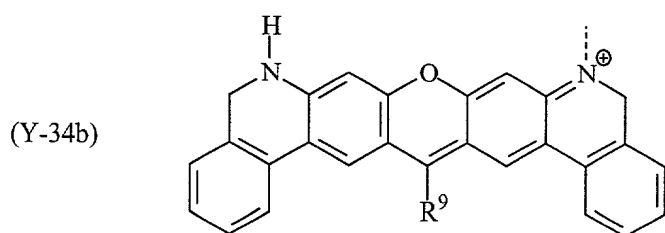
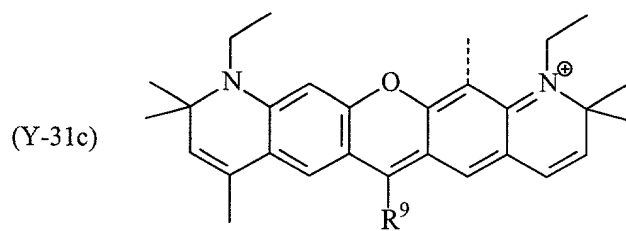
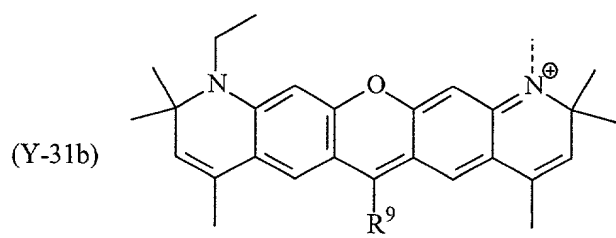
90. The labeled nucleoside/tide or nucleoside/tide analog of claim 82 wherein R^{22} , R^{23} , R^{25} , and R^{26} are each hydrogen.

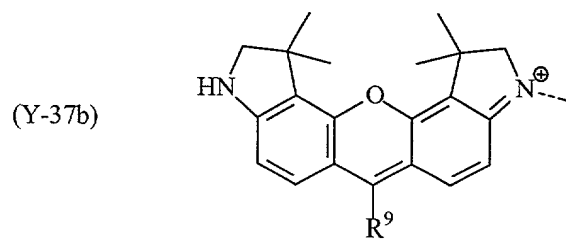
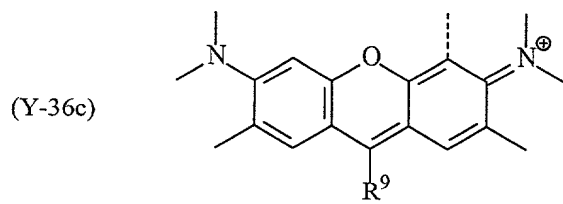
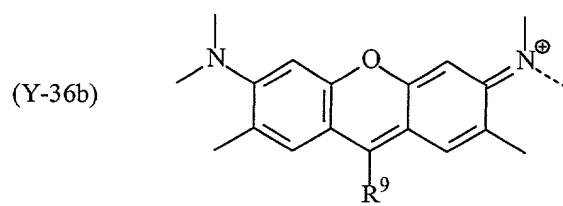
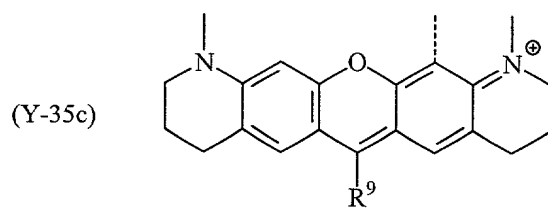
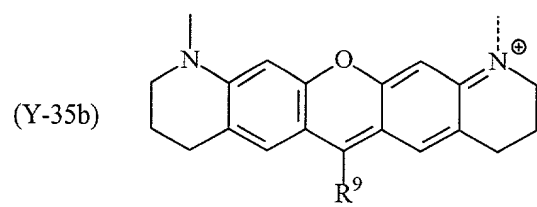
91. The labeled nucleoside/tide or nucleoside/tide analog of claim 82 in which Y^1 is selected from the group consisting of:

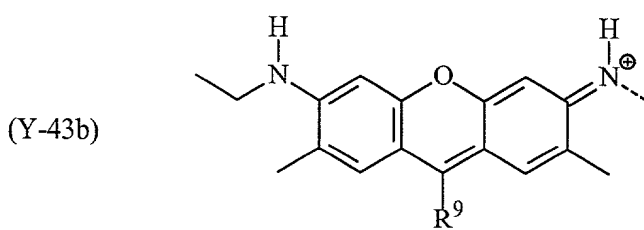
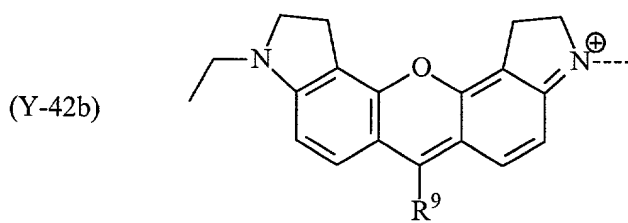
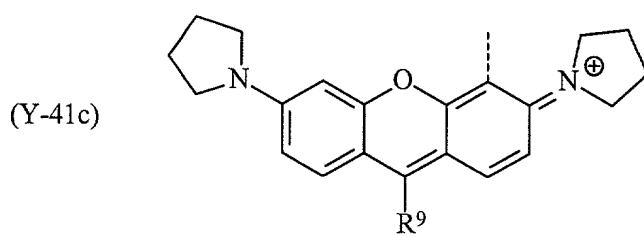
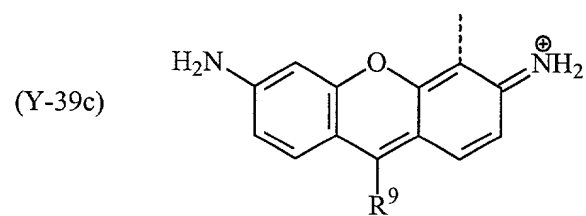
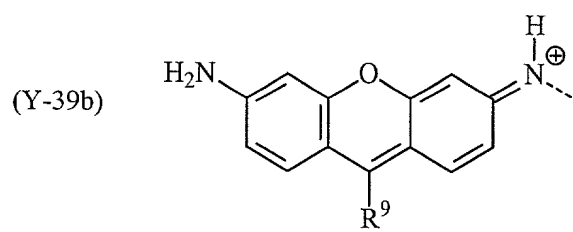


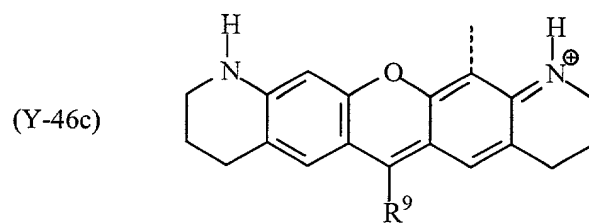
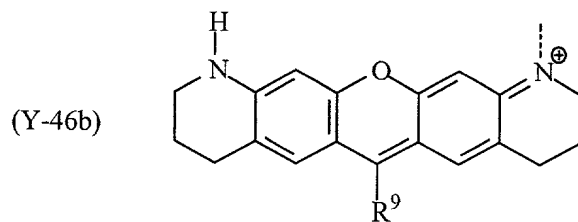
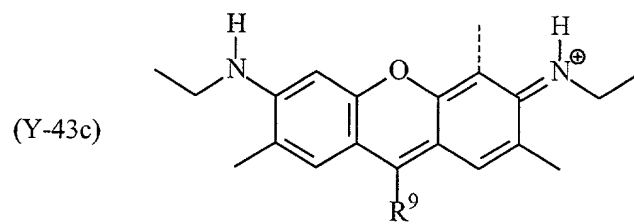






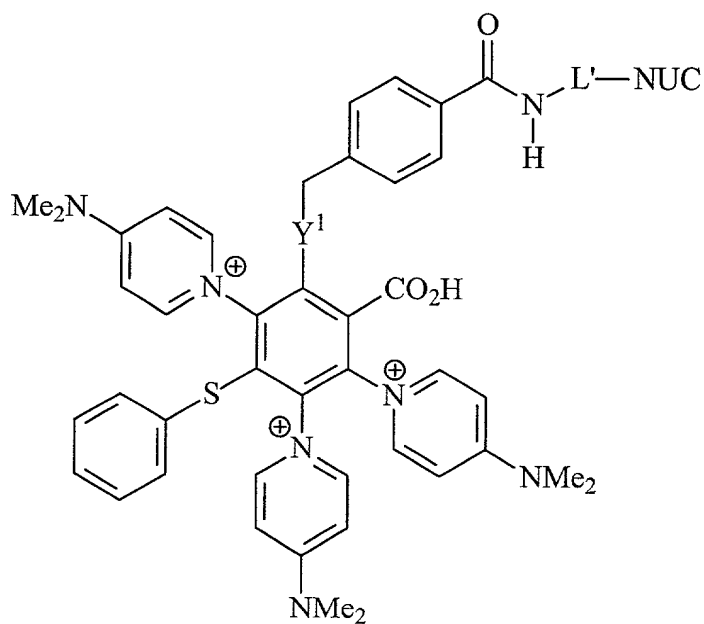






wherein the dash at the nitrogen or C4 atom indicates the point of attachment of L.

92. The labeled nucleoside/tide or nucleoside/tide analog of Claim 82 which has the structure:



93. The labeled nucleoside/tide or nucleoside/tide analog of Claim 92 in which L' is selected from: $\text{—C}\equiv\text{C—CH}_2\text{—}$ and $\text{—C}\equiv\text{C—CH}_2\text{—O—CH}_2\text{CH}_2\text{—}$.

94. The labeled nucleoside/tide or nucleoside/tide analog of Claim 70 further comprising a donor dye or an acceptor dye whereby the rhodamine dye and the donor dye or acceptor dye form an energy-transfer dye pair.

95. The labeled nucleoside/tide or nucleoside/tide analog of Claim 94 wherein the donor dye or acceptor dye is a fluorescein, rhodamine, cyanine, phthalocyanine or squaraine.

96. The labeled nucleoside/tide or nucleoside/tide analog of Claim 94 wherein the donor dye or acceptor dye is 4'-aminomethyl-6-carboxyfluorescein and the 4'-aminomethyl-6-carboxyfluorescein is covalently attached to the rhodamine dye by a linker.

97. The labeled nucleoside/tide or nucleoside/tide analog of Claim 96 wherein the aminomethylfluorescein is further covalently attached by a linker L to the nucleobase B of the nucleoside/tide or nucleoside/tide analog.

98. The labeled nucleoside/tide or nucleoside/tide analog of Claim 70 which is enzymatically incorporatable.

99. The labeled nucleoside/tide or nucleoside/tide analog of Claim 70 which is a terminator.

100. The labeled nucleoside/tide or nucleoside/tide analog of Claim 70 which is enzymatically extendable.

101. The labeled nucleoside/tide or nucleoside/tide analog of Claim 70 wherein R^{71} and R^{70} are hydrogen.

102. The labeled nucleoside/tide or nucleoside/tide analog of Claim 70 wherein R^{71} and R^{70} are hydroxyl.

103. The labeled nucleoside/tide or nucleoside/tide analog of Claim 70 wherein R^{71} is hydroxyl, and R^{70} is hydrogen.

104. The labeled nucleoside/tide or nucleoside/tide analog of Claim 70 in which nucleobase B is selected from adenine, 7-deazaadenine, cytosine, guanine, 7-deazaguanine, thymine and uracil.

105. A labeled polynucleotide or polynucleotide analog comprising a rhodamine dye conjugated to a nucleoside/tide or nucleoside/tide analog, wherein the rhodamine is a rhodamine-type parent xanthene having attached to the xanthene C9 carbon a phenyl group that is further substituted with an ortho carboxy or ortho sulfonate group or a salt thereof, one to three substituted or unsubstituted aminopyridinium groups and a substituted or unsubstituted alkylthio, or arylthio group. --

REMARKS

Entry of this Preliminary Amendment is respectfully requested. By this Amendment, claims 1-69 have been cancelled, and claims 70-105 have been added. No new matter has been added.

A restriction requirement was made in an Office Action of March 20, 2000 in parent application, Ser. No. 09/433,093. New claims 70-105 correspond to, and are consonant with Group IV, claims 46-71, drawn to labeled nucleoside/tides and including polynucleotides.

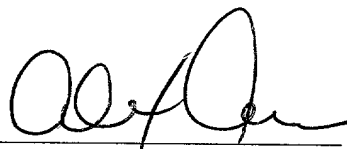
Applicants submit that the claims are in condition for allowance, and allowance is therefore respectfully requested.

If any additional fees not submitted with this response are required, the Commissioner is authorized to withdraw such fees from deposit account **01-2213**.

Respectfully submitted,

CORRESPONDENCE ADDRESS

Applied Biosystems
850 Lincoln Centre Drive
Foster City, California 94404
TEL: 650 638-5607
FAX: 650 638-6677



Alex Andrus, Ph.D.
Agent for Applicants
Reg. No. 44,509